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Thank-you!

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NEUROSCIENCE RESEARCH

COPY

Distinct spatiotemporal expression of mRNAs for the PSD-95/SAP90 protein family in the mouse brain

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Abstract

PSD-95 (SAP90), SAP102 and Chapsyn-110 (PSD-93) are members of the membrane-associated guanylate kinase family, and interact with *N*-methyl-D-aspartate (NMDA) receptor NR2A (GluRε1) and NR2B (GluRε2) subunits and with Shaker-type K^+ channel subunits to cluster into a channel complex. In the present study, we examined their expression in developing and adult mouse brains by in situ hybridization with antisense oligonucleotide probes. PSD-95 and SAP102 mRNAs were prominently expressed at embryonic day 13 (E13) in the mantle zone of various brain regions, where NMDA receptor NR2B subunit mRNA is expressed at high levels. In the early postnatal period when active synaptogenesis takes place, both mRNAs became elevated and concentrated in the telencephalon and cerebellar granular layer, where NR2A and/or NR2B subunit mRNAs are abundantly expressed. Chapsyn-110 mRNA was, though at low levels, found over the mantle zone of embryonic brains, and the level was progressively increased in the telencephalon starting at perinatal stages. The spatial and temporal correlations in the brain in vivo suggest that the PSD-95/SAP90 protein family can interact with NMDA receptor subunits to cluster them into channel complex at both synaptic and non-synaptic sites before, during and after synaptogenic stages.

Keywords: Chapsyn-110 (PSD-93); Development; In situ hybridization; Mouse; PSD-95 (SAP90); SAP102

1. Introduction

Synapses are specialized contact sites, converting electrical impulses into chemical signals to mediate information from presynaptic to postsynaptic cells. The *N*-methyl-D-aspartate (NMDA) receptor is one of three major subtypes of the glutamate receptor channel, which mediates most of the fast excitatory synaptic transmission in the mammalian central nervous system (Mayer and Westbrook, 1987). The NMDA receptor plays an essential role in the induction of activity-dependent synaptic plasticity which is thought to underlie memory and learning, and is also involved in neural development, including neuronal migration, growth cone movement, activity-dependent synapse refinement, and experience-dependent neurobehavioral development (Mattson, 1988; McDonald and Johnston, 1990). Molecularly, the NMDA receptor is heteromeric channels consisting of NR1 (GluRζ1) and NR2 (GluRε) subunits, in which the NR1 subunit has been shown to carry a glycine binding domain, while four members of NR2 (NR2A–D or GluRε1–4) subunits, glutamate binding subunits, determine the functional properties (Seeburg, 1993; Nakanishi and Masu, 1994; Mori and Mishina, 1995; Laube et al., 1997). In the brain, NR1 subunit is expressed widely from early stages of neural development, whereas each NR2 subunit displays a

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distinct expression both spatially and temporally (Watanabe, 1996).

PSD-95/SAP90 (termed PSD-95 in the present study) was originally isolated from the postsynaptic density (PSD) fraction (Cho et al., 1992; Kistner et al., 1993; Kennedy, 1997). The PDZ domain of PSD-95 binds tightly to the C-terminal tail of NMDA receptor subunits and Shaker-type K^+ channel subunits (Kistner et al., 1993; Kim et al., 1995; Niethammer et al., 1996). Successive screenings have identified three more homologous molecules, of which SAP102 and Chapsyn-110/PSD-93 (termed Chapsyn-110 in the present study) exhibit dendritic localization and clustering activity for NMDA receptors and K^+ channels (Müller et al., 1995, 1996; Kim et al., 1996). Considering the physiological importance of NMDA receptors during development and in adulthood, it is important to reveal how expression of PSD-95/SAP90 protein family is regulated. In the present study, we examined the expression of PSD-95, SAP102 and Chapsyn-110 mRNAs in the mouse brain by in situ hybridization with ^{35}S-labeled antisense oligonucleotide probes.

2. Materials and methods

2.1. Probes

Expressions of PSD-95, SAP102 and Chapsyn-110 mRNAs were investigated by in situ hybridization with 45-mer antisense oligonucleotide probes. To synthesize the mouse Chapsyn-110 probe, a partial mouse cDNA fragment corresponding to nucleotide residues 1327–1512 of the rat Chapsyn-110 cDNA (database accession number U49049) (Kim et al., 1996) was amplified by polymerase chain reaction and sequenced by Taq dideoxy terminator cycle sequencing method on an automated DNA sequencer (310 Genetic Analyzer, Perkin Elmer, Foster City, CA). Sequences of synthetic oligonucleotide probes used for data presented here are 5′-AGGGGGCGTGTCTTCATCTTGGTAGCGGTATTTCTTGGTTGTCAC-3′, 5′-CTCATAGCACTCGGGGCACTTACAGCAGTGCTGGTGCTTGTGCAT-3′ and 5′-CTCAACAGGGGAATAGTGCCTGGGAGAAGCAGGTTTATCACACAG, which are complementary to nucleotide residues 76–120 of the mouse PSD-95 cDNA (D50621), 325–369 of the mouse SAP102 (D87117), and 11–55 of the mouse Chapsyn-110 (AF069774), respectively. They were labeled with [^{35}S]dATP using terminal deoxyribonucleotidyl transferase (BRL, Gaithersburg, MD) to a specific activity of $0.5–1 \times 10^9$ dpm/μg DNA.

2.2. In situ hybridization

Under deep pentobarbital anesthesia, brains were obtained from C57BL mice at embryonic days 13 (E13), E15 and E18, postnatal days 1 (P1), P7, P14 and P21, and adult (3–4 months). The next day of overnight mating was counted as E0. Fresh frozen brain sections, cut at a thickness of 20 μm in the coronal or parasagittal plane, were mounted on glass slides precoated with 3-aminopropyltriethoxysilane. Sections were treated at room temperature with the following incubations: fixation with 4% paraformaldehyde for 10 min, 2 mg/ml glycine-phosphate-buffered saline for 10 min, acetylation with 0.25% acetic anhydride in 0.1 M triethanolamine–HCl (pH 8.0) for 10 min, and prehybridization for 1 h in a buffer containing 50% formamide, 50 mM Tris–HCl (pH 7.5), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.6 M NaCl, 0.25% sodium dodecyl sulfate (SDS), 200 μg/ml tRNA, 1 mM EDTA, and 10% dextran sulfate. Hybridization was performed at 42°C for 10 h in the prehybridization buffer supplemented with 10 000 dpm/μl of ^{35}S-labeled oligonucleotide and 0.1 M dithiothreitol. Slides were washed twice at 55°C for 40 min in 0.1 × SSC containing 0.1% sarcosyl. Sections were exposed either to Hyperfilm-β max (Amersham, Buckinghamshire, England) for 3 weeks or to nuclear track emulsion (Kodak, NTB-2, Rochester, NY) for 2 months. Emulsion-dipped sections were counterstained lightly with hematoxylin or pyronine for bright-field microscopy.

3. Results

Using consecutive parasagittal and coronal sections, PSD-95, SAP102 and Chapsyn-110 mRNAs were comparatively examined. The specificity of hybridizing signals was checked by signal disappearance when hybridization was carried out in the presence of excess unlabeled oligonucleotides, and also by similar signal patterns with use of another non-overlapping antisense probes (data not shown).

3.1. Distribution in adult mouse brain

Overall expression in the adult brain was examined by X-ray film macroautoradiography with parasagittal sections (Fig. 1A–C). PSD-95, SAP102 and Chapsyn-110 mRNAs were all expressed at high levels in the telencephalon, where the pyramidal cell layer of the hippocampus displayed the highest expression. High levels of PSD-95 and SAP102 mRNAs were also detected in the cerebellar cortex, with low to moderate signals in various thalamic and brainstem regions (Fig. 1A,B). On the other hand, Chapsyn-110 mRNA was expressed at moderate levels in the Purkinje cell (PC) layer of the cerebellum and at low levels in the hypothalamus and brainstem, whereas few signals were found in the thalamus (Fig. 1C).

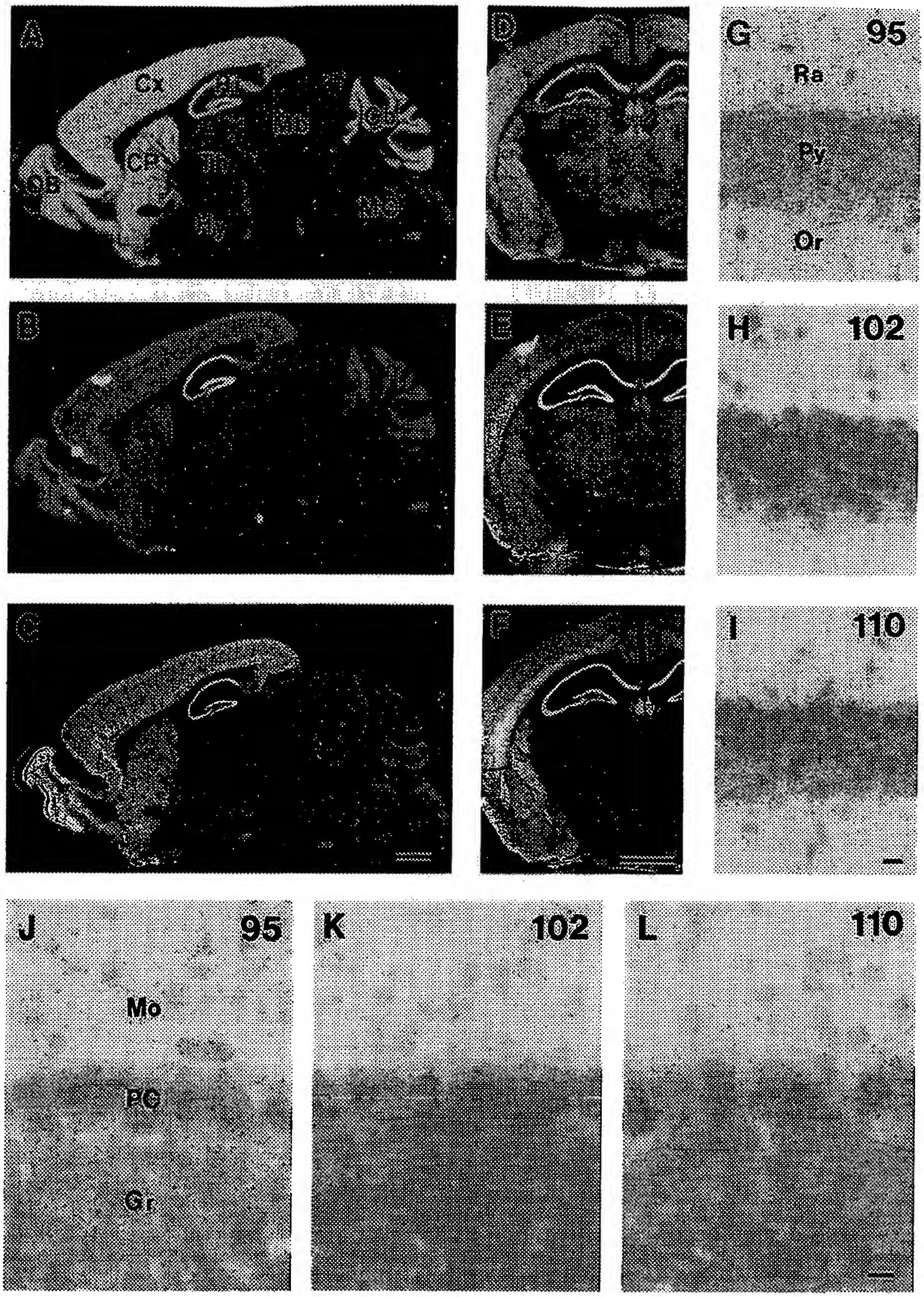

Fig. 1.

Their distributions were examined in more detail by emulsion microautoradiography with coronal sections (Fig. 1D–L, Fig. 2 and Table 1). In most telencephalic regions, all three mRNAs were distributed at high levels in the mitral and granule cell layers of the olfactory bulb, laminae II–VI of the cerebral cortex, pyramidal and granule cell layers of the hippocampus, caudate–putamen, and lateral septum (Fig. 1D–I). In the hippocampus, signals for respective mRNAs were concentrated in cell bodies of the pyramidal cells (Fig. 1G–I). In other brain regions, PSD-95, SAP102 and Chapsyn-110 mRNAs were widely distributed at low to moderate levels, showing different relative abundance depending on nuclei and neuron types. In the thalamus, PSD-95 and SAP102 mRNAs were expressed at moderate to low levels in various nuclei, whereas Chapsyn-110 mRNA was restricted to the medial habenula (Fig. 1D–F). In the brainstem, moderate signals for PSD-95 mRNA were associated with various nuclei, such as motor nuclei (trigeminal motor nucleus, facial nucleus, dorsal motor nucleus of the vagus and hypoglossal nucleus) and precerebellar nuclei (reticulotegmental nucleus, pontine nucleus, inferior olive and lateral reticular nucleus) (Fig. 2A,D,G, and Table 1). On the other hand, SAP102 mRNA was particularly abundant in the precerebellar nuclei (Fig. 2B,E,H and Table 1), whereas Chapsyn-110 mRNA was concentrated in the motor nuclei and dorsal raphe nucleus (Fig. 2C,F,I and Table 1). In the cerebellum, all three mRNAs were more or less observed in cell bodies of PCs and granule cells; signal intensities in PCs and granule cells were almost comparable for PSD-95 and SAP102 mRNAs, while Chapsyn-110 mRNA in PCs was apparently higher than that in granule cells (Fig. 1J–L). Neuronal cell bodies in the molecular layer, which represent basket or stellate neurons, were labeled for PSD-95 and SAP102 mRNAs, but labeling was obscure for Chapsyn-110 mRNA due to low signal levels (Fig. 1J–L).

In given regions examined by bright-field microscopy, labelings for PSD-95, SAP102 and Chapsyn-110 mRNAs were generally seen in larger cell bodies having pale nuclei, but rare or absent in small cells with dark nuclei, suggesting neuronal expression. In addition, silver grains representing PSD-95 mRNA were detected diffusely over the neuropil of the strata oriens and radiatum in the hippocampus (Fig. 1A,G) and of the molecular layer in the cerebellum (Fig. 1A,J), suggesting its dendritic localization.

3.2. Developmental changes

Developmental changes were followed from E13 to P21 by X-ray film macroautoradiography with parasagittal brain sections (Fig. 3). A prominent expression of PSD-95 mRNA was detected as early as E13 in the mantle zone of various brain regions, but not in the ventricular zone (Fig. 3A–G). The wide expression in the mantle zone/gray matter was maintained throughout development, exhibiting a'V'-shaped change in the intensity with a trough at P1 or P7. Thereafter, the intensity displayed a remarkable increase, particularly in the telencephalon and cerebellum, reaching the peak level at P14–P21. A temporal profile of SAP102 mRNA expression was almost similar to that of PSD-95 mRNA during the embryonic period, but postnatal increase of the former was not so marked compared to that of the latter (Fig. 3H–N). Chapsyn-110 mRNA was detectable widely in the mantle zone at E13 and E15, but the level was considerably low (Fig. 3O,P). High levels of Chapsyn-110 mRNA appeared in the telencephalon starting at E18 and P1, and the intensity progressively increased thereafter reaching the peak level at P14–P21 (Fig. 3Q–U). In other brain regions, levels of Chapsyn-110 mRNA were consistently low from E18 to P21.

4. Discussion

Our present results on high levels of PSD-95 mRNA in the telencephalon and cerebellar cortex and on abundant expression of Chapsyn-110 mRNA in cerebellar PCs are consistent with previous results in the adult rat brain by in situ hybridization (Kornau et al., 1995; Brenman et al., 1996a,b; Kim et al., 1996) and by immunohistochemistry (Kistner et al., 1993; Kim et al., 1995; Hunt et al., 1996). Wide expression of PSD-95 and Chapsyn-110 mRNAs in the mantle zone of embryonic brains is also parallel with previous in situ hybridization applied to the rat brain at E15 (Brenman et al., 1996a). The present analysis has further provided detailed and comparative information on the spa-

Fig. 1. Distributions of PSD-95 (A, D, G, J), SAP102 (B, E, H, K) and Chapsyn-110 (C, F, I, L) mRNAs in the adult mouse brain. (A–C) X-ray film macroautoradiographs of parasagittal sections. (D–F) Dark-field micrographs of coronal sections through the hippocampus and diencephalon. (G–L) Bright-field micrographs of CA1 region in the hippocampus (G–I) and cerebellar cortex (J–L). Am, amygdala; CA1, CA3, CA1 and CA3 regions of the hippocampus; Cb, cerebellum; CP; caudate–putamen; Cx, cerebral cortex; DG, dentate gyrus; Gr, granular layer; Hb, habenula; Hi, hippocampus; Hy, hypothalamus; LG, lateral geniculate nucleus; Mb, midbrain; Mo, molecular layer; OB, olfactory bulb; Or, stratum oriens; PC, Purkinje cell layer; Py, pyramidal cell layer; Ra, stratum radiatum; Th, thalamus; VM, ventromedial hypothalamic nucleus; VPM/L, ventral posteromedial and posterolateral nuclei; 1–6, laminae I–VI of the cerebral cortex. Scale bars: 1 mm (C, F) 10 μm (I, L).

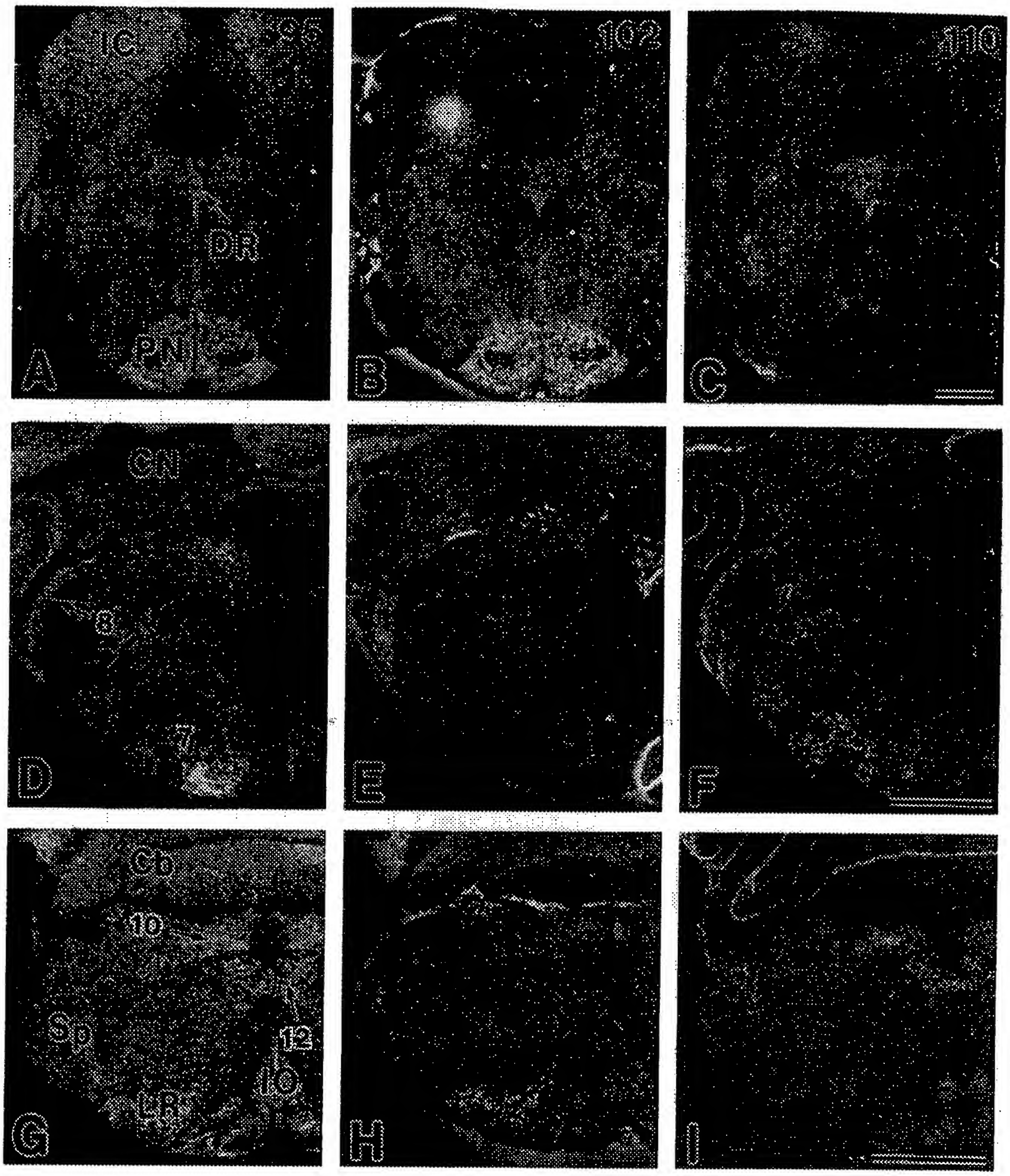

Fig. 2. PSD-95 (A, D, G), SAP102 (B, E, H) and Chapsyn-110 (C, F, I) mRNAs in the brainstem. Emulsion-dipped coronal sections through the midbrain–pons junction (A–C), pons (D–F), and medulla oblongata (G–I).

tiotemporal expression of PSD-95, SAP102 and Chapsyn-110 mRNAs.

The PDZ domain of the PSD-95/SAP90 protein family binds specifically and tightly to the C-terminal tail having a S/TXV motif. Although the motif is shared with NMDA receptor NR2A–D subunits and some

NR1 splice variants (NR1–3 and NR1–4), transfection and immunoprecipitation studies have revealed that PSD-95, SAP102 and Chapsyn-110 actually interact with NR2A and NR2B subunits (Kornau et al., 1995; Kim et al., 1996; Lau et al., 1996; Müller et al., 1996; Niethammer et al., 1996). In the embryonic brain, NR2B subunit is highly expressed in the mantle zone of various brain regions; in mice it is detectable as early as E13 (Watanabe et al., 1992). NR2B subunit disappears from the cerebellum and brainstem during the early postnatal period, and becomes concentrated in the telencephalon and thalamus at the adult stage (Watanabe et al., 1992, 1993; Akazawa et al., 1994; Monyer et al., 1994; Watanabe et al., 1994). The gene knockout strategy has revealed that NMDA receptors containing NR2B subunit play an essential role in the development of somatosensory synapses and in the induction of long-term depression in neonatal hippocampus (Li et al., 1994; Kutsuwada et al., 1996). In contrast, NR2A subunit appears postnatally and is distributed widely in the adult brain, with higher levels in the telencephalon and cerebellar granule cells. Both NR2A and NR2B subunits are expressed at the highest level in adult hippocampus (Watanabe et al., 1993, 1998), where NMDA receptors are involved in hippocampal LTP induction and spatial learning (Sakimura et al., 1995; Ito et al., 1997). It should be noted that members of the PSD-95/SAP90 protein family are expressed in all of these regions where NMDA receptor subunit mRNAs are present. Therefore, developing and mature neurons in vivo may co-express PSD-95/SAP90 protein family and NMDA receptor subunits. The correlated spatiotemporal expressions would be significant for the formation and clustering of receptor channel complex at both synaptic and non-synaptic sites before, during and after synaptogenic stages. Considering that NMDA receptor subunit composition changes dynamically during the first 2 postnatal weeks of a rodent's life (Watanabe et al., 1992; Akazawa et al., 1994; Monyer et al., 1994), two elevated transcriptions of the PSD-95/SAP90 protein family, first during the fetal period and second at early postnatal stages, might suggest their enhanced requirements to tailor NMDA receptor subunits into fetal- or adult-type receptor complex, respectively.

Most central neurons co-express NR1 and NR2 subunits, both being indispensable for the formation of functional NMDA receptors. Despite high levels of NR1 subunit, none of the NR2 subunits are expressed in adult PCs (Watanabe et al., 1994), where NMDA receptor-mediated postsynaptic responses are not recorded and the induction of synaptic plasticity does not depend on NMDA receptor activation (Aiba et al., 1994; Conquet et al., 1994; Kashiwabuchi et al., 1995). However, PCs express PSD-95, SAP102 and Chapsyn-110 mRNAs at substantial levels. The dissimilarity might be reflected partly by the presence of other molecules to which the PSD-95/SAP90 protein family bind, such as Shaker-type K^+ channel subunits (Kues and Wunder, 1992; Veh et al., 1995; Laube et al., 1996).

Different relative abundance of PSD-95, SAP102 and Chapsyn-110 mRNAs is noted in some neural regions and also during development. The three molecules are structurally conserved, sharing three PDZ domains, Src homology region 3 (SH3) domain and guanylate kinase domain, but their functional differences have not yet been elucidated. Future studies will be of great interest to address whether the different transcriptional regulations represent their different spectrum of substrate molecules to be clustered in given neurons.

Table 1
Relative abundance[a] of PSD-95, SAP102 and Chapsyn-110 mRNAs in adult mouse brain

Region	PSD-95	SAP110	Chapsyn-110
Olfactory bulb[b]	+++	++	+++
Cerebral cortex	+++	++	++
Hippocampus			
Pyramidal cell layer	++++	++++	++++
Granule cell layer	+++	++++	++++
Caudate–putamen	+++	++	++
Septum (lateral nucleus)	+++	++	++
Amygdala	+++	++	++
Thalamus			
VPM/VPL, LGN, MGN[c]	++	++	–
Medial habenula	++	++	+++
Hypothalamus			
Paraventricular nucleus	+	+	–
Ventromedial nucleus	++	+	+
Superior colliculus	++	+	+
Inferior colliculus	++	+	+
Substantia nigra	+	+	+
Precerebellar nuclei			
Pontine nuclei	++	+++	+
Reticulotegmental nucleus	++	+++	+
Lateral reticular nucleus	++	+++	–
Inferior olive	++	+	+
Dorsal raphe nucleus	+	+	++
Motor nuclei[d]	++	–	++
Somatosensory (trigeminal)	+	+	+
Cochlear nucleus (dorsal)	++	+	++
Cerebellum			
Purkinje cell	++	++	+++
Granular layer	++	++	+
Basket/stellate cell	++	+	–
Deep cerebellar nuclei	+	+	+

[a] Relative expression levels were estimated by visual comparison of emulsion-dipped coronal sections in the dark-field view. –, not detected; +, low; ++, moderate; +++, high; ++++, very high.
[b] Mitral and granule cell layers.
[c] Ventral posteromedial/posterolateral nuclei, lateral geniculate nucleus, and medial geniculate nucleus.
[d] Trigeminal motor nucleus, facial nucleus, dorsal motor nucleus of the vagus, and hypoglossal nucleus.

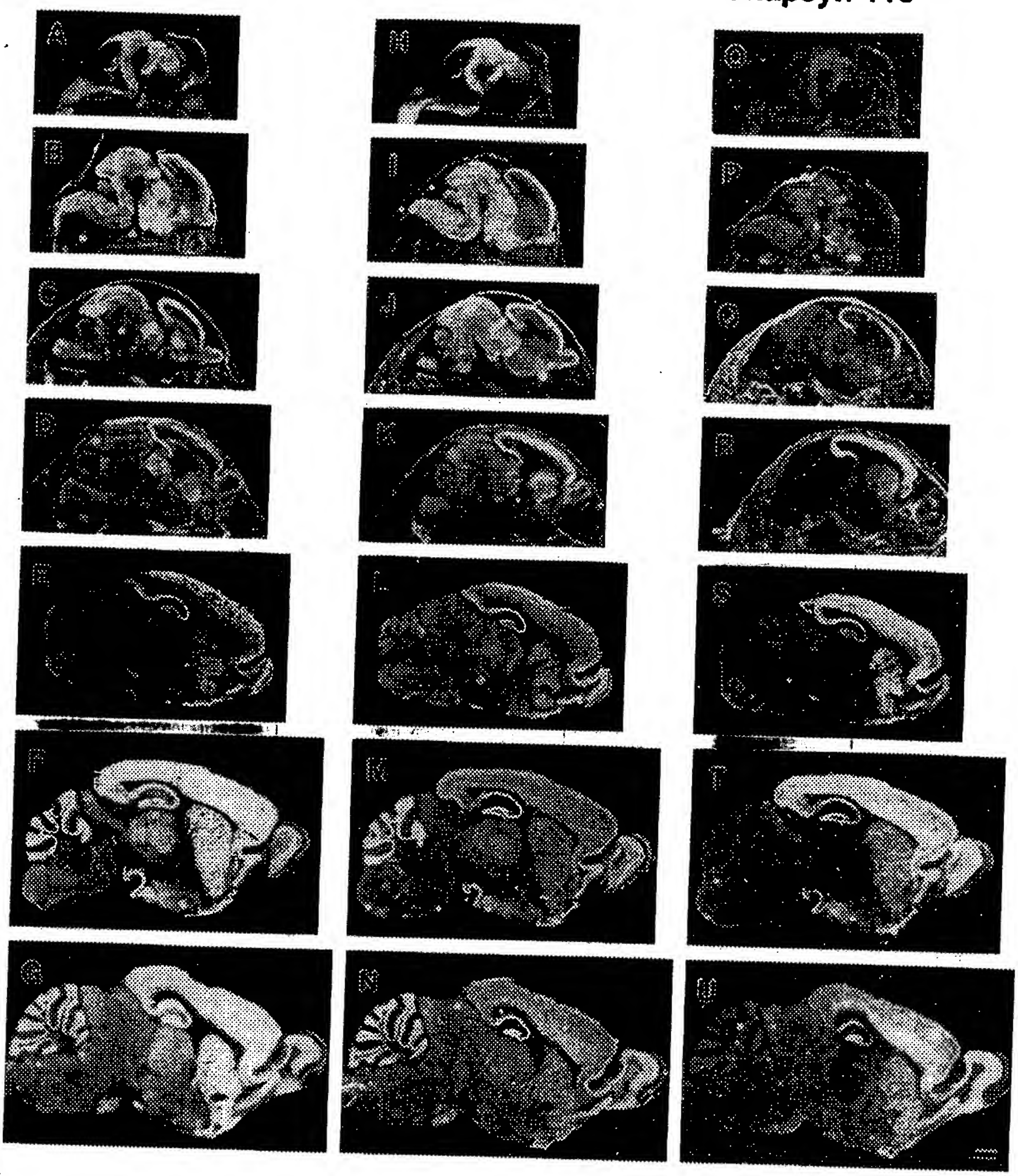

Fig. 3. Developmental changes in expressions for PSD-95 (A–G), SAP102 (H–N) and Chapsyn-110 (O–U) mRNAs in mouse brain. E13 (A, H, O); E15 (B, I, P); E18 (C, J, Q); P1 (D, K, R); P7 (E, L, S); P14 (F, M, T); P21 (G, N, U). Each set of hybridized sections was exposed to a single X-ray film, from which photographs were directly printed at the same magnification. The rostral is to the right, and the dorsal is to the top. Scale bar: 1 mm.

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